DOI: 10.5433/1679-0359.2025v46n3p739

Impact of doses of protected fat supplementation on milk composition and performance of lactating Saanen goats

Impacto de doses de suplementação de gordura protegida na composição do leite e no desempenho de cabras Saanen lactantes

Rodrigo de Souza[1]; Claudete Regina Alcalde[2]; Júlio Cesar Damasceno[3]; Ludmila Couto Gomes Passetti[4]; Bruna Susan de Labio Molina[5]; Bruna Hygino[6]; José Augusto Horst[7]; Geraldo Tadeu dos Santos[8]*

Highlights

Supplementation with protected fat improved nutritional efficiency in goats.
Production varied with lactation and correlated positively with dietary energy levels.
Energy affected the milk fatty acid profile: C18:1n9t, C18:2n6c, and C18:3n3.
Meeting nutritional needs during lactation is vital for milk production and quality.

Abstract

This study explored the effects of protected fat supplementation on energy intake, nutrient efficiency, blood parameters, and milk production in lactating Saanen goats. Twenty multiparous goats were assigned to four diets with increasing energy levels (2.6, 2.7, 2.8, and 2.9 Mcal ME•kg^{-1} DM), achieved by including protected fat in the diet as calcium salts of fatty acids (CSFA). Our findings revealed no

[1] Prof. Dr. of Instituto Federal do Paraná, IFPR, Ensino Básico, Técnico e Tecnológico, EBTT, Campus Campo Largo, Campo Largo, PR, Brazil. E-mail: rodrizoo15@gmail.com
[2] Profª Drª of the Postgraduate Program in Animal Science (PPZ), Universidade Estadual de Maringá, UEM, Maringá, PR, Brazil. E-mail: cralcalde@uem.br
[3] Prof. Dr. of the Postgraduate Program in Animal Science (PPZ), UEM, Maringá, PR, Brazil. E-mail: jcdamasceno@uem.br
[4] Profª Drª of Universidade Federal dos Vales de Jequitinhona e Macuri, UFVJM, Campus de Unaí, MG, Brazil. E-mail: ludmilacoutogomes@gmail.com
[5] Postgraduate Student of the master's Program in Animal Science of UEM, Maringá, PR, Brazil. E-mail: brunamolina_@hotmail.com
[6] Postgraduate Student of the master's Program in Animal Science of UEM, Maringá, PR, Brazil. E-mail: bru_hy@hotmail.com
[7] Researcher in Charge of the Milk Analysis Laboratory, PARLEITE da Associação Paranaense dos Criadores de Bovinos da raça Holandesa (APCBRH), Curitiba, PR, Brazil. E-mail: horst@apcbrh.com.br;
[8] Prof. Dr. of the Postgraduate Program in Animal Science, Universidade Federal de Mato Grosso do Sul, UFMS, Campo Grande, MS, Brazil. E-mail: gtsantos50@gmail.com
* Author for correspondence

Received: Oct. 08, 2024 Approved: Mar. 25, 2025

impact on dry matter intake, while ether extract and total digestible nutrient intakes increased, and non-fibrous carbohydrate intake decreased linearly. Enhanced digestibility of key nutrients indicated improved overall dietary efficiency. Blood cholesterol and plasma urea levels increased in response to higher dietary energy levels. Milk production varied with the lactation phase, showing a positive and linear relationship with dietary energy initially (Y = -3.76 + 2,71X; R^2=0.24) and a quadratic response from 121 days until 181 days in lactation (Y = -217.60 + 157.38X -28,03X^2; R^2=0.62) affecting milk fat, lactose, and protein content. Fatty acid analysis showed both linear and quadratic responses among different types, highlighting the crucial role of dietary energy in modulating the milk fatty acid profile for improved nutritional quality. Beneficial long-chain fatty acids like C18:0 and C18:3n3 increased, while short-chain and medium-chain fatty acids declined with higher energy levels. Additionally, C18:2n6c and C20:1 showed significant quadratic responses. These findings suggest the lactation phase-dependent effects of protected fat supplementation on milk production and composition, emphasizing the importance of meeting specific nutritional demands throughout lactation.
Key words: Calcium salts of fatty acids. Dairy goats. Milk composition. Milk production.

Resumo

Este estudo explorou os efeitos da suplementação de gordura protegida sobre a ingestão de energia, eficiência dos nutrientes, parâmetros sanguíneos e produção de leite em cabras Saanen lactantes. Vinte cabras multíparas foram distribuídas em quatro dietas com níveis crescentes de energia (2,6, 2,7, 2,8 e 2,9 Mcal ME•kg^{-1} DM), obtidos pela inclusão de gordura protegida na dieta como sais de cálcio de ácidos graxos. Os resultados revelam que não houve impacto no consumo de matéria seca, enquanto o consumo de extrato etéreo e nutrientes digestíveis totais aumentou, e a ingestão de carboidratos não fibrosos diminuiu linearmente. A maior digestibilidade de nutrientes-chave indicou uma melhoria na eficiência dietética geral. Os níveis de colesterol sanguíneo e ureia plasmática aumentaram em resposta ao incremento de níveis de energia na dieta. A produção de leite variou com a fase de lactação, mostrando inicialmente relação linear positiva com a energia dietética (Y = -3.76 + 2,71X; R^2 = 0,24) e resposta quadrática posteriormente aos 121 dias até 180 em lactação (Y = -217.60 + 157.38X -28,03X^2; R^2 = 0,62), afetando os teores de gordura, lactose e proteína do leite. A análise de ácidos graxos mostrou respostas lineares e quadráticas entre diferentes tipos, destacando o papel crucial da energia dietética na modulação do perfil de ácidos graxos do leite para uma melhor qualidade nutricional. Ácidos graxos de cadeia longa benéficos, como C18:0 e C18:3n3, aumentaram, enquanto ácidos graxos de cadeia curta e média diminuíram com níveis mais altos de energia. Além disso, C18:2n6c e C20:1 apresentaram respostas quadráticas significativas. Esses achados revelam efeitos dependentes da fase de lactação da suplementação de gordura protegida na produção e composição do leite, enfatizando a importância de atender às demandas nutricionais específicas ao longo da lactação.
Palavras-chave: Cabras leiteiras. Composição do leite. Produção de leite. Sais de cálcio de ácidos graxos.

Introduction

Lipid supplementation in ruminant diets has emerged as a key strategy for enhancing energy supply during high-demand periods and modulating milk composition and nutritional quality, with significant implications for animal performance and human health. The introduction of calcium salts of fatty acids (CSFA) represents an advancement, allowing for greater dietary inclusion without compromising fiber digestibility. CSFAs interact with calcium ions and long-chain fatty acids to form a rumen-resistant complex that dissociates in the acidic abomasum for absorption, demonstrating the importance of these protected fats in ruminant nutrition and performance (Cappellozza et al., 2020).

Granados-Rivera et al. (2020) highlight the dual benefits of lipid supplementation in enhancing energy balance and increasing milk production, particularly during the early lactation phase in goats. This observation is crucial for understanding lipid supplementation's role as a targeted nutritional intervention to optimize productivity and support metabolic health in lactating goats. Furthermore, Shpirer et al. (2023) explore the digestibility and impact of different fat supplements, including CSFA, on feed efficiency, indicating that calcium salts of fatty acids are more digestible than free fatty acids and have differential effects on milk yield and fat concentration in dairy cows.

The manipulation of lipid sources such as CSFA or dietary supplementation with flaked linseed or fish oil can significantly impact milk fat content, protein composition, and the fatty acid profile of milk and cheese in ruminants. Research by Casals et al. (2006) and Savoini et al. (2019) has shown that CSFA supplementation increased milk fat content in dairy ewes and goats, with potential implications for milk quality and health status. Similarly, Schroeder et al. (2004) noted that fat supplementation in grazing dairy cows increased milk production, with saturated fat sources leading to higher milk fat concentration.

Despite these advancements, there are still gaps in understanding the optimal lipid supplementation strategies, particularly regarding the optimal use of CSFA supplementation in goat diets. The variability in responses to lipid supplementation, influenced by many factors, necessitates further research to elucidate the underlying mechanisms and optimize supplementation strategies (Souza et al., 2014; Del Valle et al., 2021; Moya et al., 2023).

Therefore, we hypothesize that increasing dietary energy levels through CSFA supplementation will improve nutrient utilization efficiency, influence the fatty acid profile of milk, and enhance milk production performance in Saanen goats without negatively affecting dry matter intake or metabolic parameters. Addressing these gaps, this study aimed to investigate the effects of dietary energy levels, through CSFA supplementation, on the nutritional value of diets, milk composition, and milk production of Saanen goats after 21 days in milk.

Materials and Methods

All experimental procedures were conducted in accordance with applicable ethical guidelines and the recommendations of the local Animal Ethics Committee of

the State University of Maringá, in Maringá, Paraná, Brazil (CEUA no. 2217210519). The experiment was conducted in Maringá, state of Paraná, Brazil (geographical coordinates 23˚25′ S; 51˚57′ W, 540 m above sea level). Twenty Saanen multiparous goats (body weight: 63.5 ± 10.3 kg; milk yield: 3.3 kg^{-1} day) were assigned to four treatments in a completely randomized design. To minimize variation among treatments, the animals were balanced according to body weight and lactation number (2nd, 3rd, and 4th lactation) before treatment assignment. The four treatments consisted of diets with increasing energy levels obtained by the addition of CSFA (Table 1).

Table 1
Proportion of ingredients and chemical composition of the diets

Food (g kg^{-1} DM)	Diets (Mcal of EM kg^{-1} of DM)			
	2.6	2.7	2.8	2.9
Corn silage	574.8	600.0	600.0	600.0
Ground corn	244.4	185.7	154.1	122.6
Soybean meal	172.6	179.5	184.9	190.2
Protected fat (Lactoplus®)	-	28.7	54.6	80.5
Limestone	1.2	-	-	-
Dicalcium phosphate	-	1.1	1.3	1.6
Mineral-vitamin supplement[1]	7.0	5.0	5.0	5.0
Dry matter (DM)	528.6	513.7	517.2	518.1
Organic matter (OM)	951.6	949.5	944.7	941.8
Crude protein (CP)	156.9	158.8	160.6	164.4
Ethereal extract[3](EE)	28.2	26.5	25.5	24.6
Supplemental fat[4] (SF)	-	23.6	44.8	66.0
Neutral detergent fiber (NDF)	349.8	359.4	344.9	341.7
Non-fibrous carbohydrates	416.8	381.3	368.8	344.9
Total carbohydrates (TC)	766.6	740.7	713.7	686.7

[1]Chemical composition (per kg of product): calcium 240 g; phosphorus 71 g; fluorine 710 mg (max.); magnesium 20 g; potassium 28.20 g; vit. A 135,000 IU; vit. D3 68,000 IU; vit. E 450 IU; iron 2,500 mg; copper 400 mg; manganese 1,350 mg; zinc 1,700 mg; cobalt 30 mg; iodine 40 mg; selenium 15 mg; chromium 10 mg; 95% Solubility of phosphorus in citric acid 2% (Min). [2]g/kg as fed; [3]Obtained from the analysis of corn silage, ground corn, and soybean meal; [4]Estimated based on information from the manual from the Lactoplus® manufacturer (Dalquim Indústria Química Ltd.).

The control diet had 2.6 Mcal ME/kg dry matter (DM) and was formulated according to the National Research Council [NRC] (2007) requirements for Saanen goats with 60 kg of body weight, producing 3.0 kg of milk per day. In the remaining treatments, fat was added in the form of CSFA, using a commercial product based on soybean oil (Lactoplus®, Dalquim Chemical Industry Ltd.), to obtain the following energy levels: 2.7; 2.8; and 2.9 Mcal ME•kg^{-1} DM (Table 1).

The experimental period was from 21 days after birth until 180 days in milk; nevertheless, the animals received the diets starting from the last 35 days of pregnancy. Animals were housed in individual stalls with *ad libitum* water and access to a solarium in the morning for 2 h. Goats were fed twice daily, at 09:30 and 14:30 with a total mixed ration offered in two meals. Feed and orts were measured daily, and feed intake was adjusted weekly to allow for approximately 10% orts. Milking was performed manually twice daily at 07:30 and 15:00, followed by milk weighing.

A sampling week was conducted at 90 days in milk. Body weight (BW), dry matter intake (DMI), and nutrient digestibility were measured. DMI was calculated as the difference between the feed supplied and the orts during the sampling week.

To determine DM and nutrient digestibility, fecal samples were collected from the animals' rectum for six days at different times each day (08:00, 10:00, 12:00, 14:00, 16:00, and 18:00), to obtain a combined sample for each animal. Fecal, feed, and orts samples were frozen at -20 °C for further analysis.

For analysis, samples were defrosted, dried in a forced-air oven at 55 °C for 72 h, ground in a knife mill to pass through a 1-mm screen sieve and stored in plastic bottles. Fecal excretion was estimated by using indigestible neutral detergent fiber (iNDF) as an internal marker, according to the adapted methodology from Cochran et al. (1986). The iNDF was obtained after 144 h of incubation *in situ* (fistulated goats) of feed, orts, and feces in an F57 filter (Ankom® Technology Corporation), followed by neutral detergent fiber (NDF) analysis.

Dry matter (DM; method 950.15), mineral matter (MM; method 942.05), crude protein (CP; Kjeldahl method 984.13), and ether extract (EE; method 920.39) were analyzed according to the Association on Official Analytical Chemists [AOAC International] (2000). Neutral detergent fiber (NDF) concentrations were determined according to Van Soest et al. (1991) using sodium sulfite and α-amylase, with a fiber determiner (TE-149, Tecnal, São Paulo, Brazil). Organic matter (OM) content was calculated using the formula OM (g/kg) = 1000 - MM.

The values of total carbohydrates (TC) and total digestible nutrients (TDN) were estimated according to the equations described by Sniffen et al. (1992): TC (g/kg DM) = 1000 – (CP + EE + ash); and TDN = dCP + (2.25 x dEE) + dTC; where: dCP = digestible crude protein, dEE = digestible ether extract, and dTC = digestible total carbohydrates. Nonstructural carbohydrate (NSC) values were estimated according to the equation described by Van Soest et al. (1991): NSC (g/kg DM) = 1000 – (NDF + CP + EE + ash).

Milk production was measured daily, totaling two milkings per day. Proportional milk samples were collected monthly from each animal during both milking times. Samples were packed in plastic bottles containing Bronopol® (2-bromo-2-nitropropane-1,3-diol) and analyzed for fat, protein, lactose, and total solids by infrared spectroscopy using a Bentley 2000® analyzer (Bentley Instrument Inc., Chaska, MN). Milk somatic cell count (SCC) was determined by flow cytometry using a Somacount 500® analyzer (Bentley Instruments Inc., Chaska, MN, USA), calibrated for cow milk analysis but also validated for goat milk analysis, according to Andrade et al. (2008). Analyses were conducted at the Laboratório de Análise de Leite – PARLEITE of the Associação Paranaense dos Criadores de Bovinos da raça Holandesa (APCBRH) in Curitiba, Paraná.

To approximate the SCC to a normal distribution with variance homogeneity, SCC was converted into somatic cell score (SCS) using the equation described by Ali and Shook (1980): $SCS = \log_2 (SCC/100,000) + 3$.

Blood samples were collected at 90 days in milk, after morning milking (before feeding), via jugular vein puncture. Samples were placed in 10-mL test tubes and centrifuged at 3,500 rpm for 15 min to obtain serum. The serum was stored in Eppendorf tubes and analyzed using a Vitalab Selectra-2® autoanalyzer with Merck diagnostic kits for cholesterol, triglycerides, and glucose at the UEM Biochemistry Laboratory.

The same methodology for blood collection, serum processing, and storage was applied at 90 days in milk to determine the average urea levels during the experimental period. Analyses were carried out using the enzymatic-colorimetric method with the Urea-PP kit, category 427 (Analisa®).

Milk samples were collected for FA concentration analysis of milk fat, and blood was collected for analysis of biochemical composition analysis. Milk fat was separated by centrifugation as described by Murphy et al. (1995), and FA was methylated according to method 5509 of International Organization for Standardization [ISO] (1978) using KOH/methanol and n-heptane. FA methyl ester concentrations were measured using a gas chromatography system (GC Ultra Trace, Thermo Scientific) equipped with a flame ionization detector (at 240 °C), a fused silica capillary column detector, and a CP-7420 fused silica capillary column (100 m and 0.25 mm i.d., 0.25-m film thickness). Fatty acids were quantified by comparing retention times with FA methyl ester standards (Sigma Aldrich®).

The effects of dietary energy levels on BW, feed intake, digestibility, milk composition, blood biochemical profile, and milk fatty acid profile were evaluated using linear and quadratic regressions with the statistical software SPSS (Version 18). The best-fit model was selected based on the Tukey test ($P<0.05$).

Results

Dry matter intake (DMI), with an average of 2.05 kg/day, was not affected by treatments. Similarly, there was no effect of treatments on the intake of organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF) (Table 2). The intake of EE and TDN increased dietary energy levels linearly, while NFC intake decreased linearly.

Table 2
Body weight (BW), intake, and digestibility of dry matter and nutrients at 90 days in milk in Saanen goats receiving diets with increasing energy levels achieved by adding protected fat

Variable	Diets (Mcal of EM kg^{-1} of DM)				Regression equation; R^2		CV (%)
	2.6	2.7	2.8	2.9			
BW (kg)	63.32	62.88	63.42	62.30	Y = 62.98;	ns[1]	18.88
Intake (kg^{-1} day)							
DM	2.08	2.05	1.99	2.07	Y = 2.05;	ns	10.71
DM_{BW}[2]	33.94	34.09	31.50	33.24	Y = 33.19;	ns	16.75
OM	1.97	1.94	1.87	1.94	Y = 1.93;	ns	10.67
CP	0.33	0.34	0.34	0.35	Y = 0.34;	ns	11.15
EE	0.06	0.11	0.15	0.21	Y = -1.260+0.508X;	0.88	15.42
NDF	0.68	0.69	0.63	0.62	Y = 0.66;	ns	12.40
TC	1.58	1.49	1.37	1.37	Y = 1.45;	ns	10,25
NFC	0.90	0.80	0.74	0.75	Y = 1.933-0.412X;	0.27	9.62
TDN	1.49	1.40	1.61	1.69	Y = -0.595+0.799X;	0.20	11.71
Digestibility coefficient (kg^{-1} kg)							
DM	0.71	0.64	0.74	0.72	Y = 0.70;	ns	4.87
OM	0.72	0.66	0.76	0.74	Y = 0.72;	ns	4.55
CP	0.72	0.67	0.79	0.77	Y = 0.002+0.268X;	0.34	3.21
EE	0.88	0.91	0,95	0.96	Y = 0.210+0.259X;	0.75	1.89
NDF	0.53	0.39	0,53	0.45	Y = 0.47;	ns	8.66
TC	0.72	0.64	0,73	0.70	Y = 0.70;	ns	5.26
NFC	0.86	0.85	0.90	0.90	Y = 0.377+0.182X;	0.23	4.45
TDN	0.72	0.68	0.81	0.82	Y = -0.377+0.412X;	0.53	4.25

[1]ns = P>0.05; [2]g/kg of body weight; R^2 = coefficient of determination; CV = coefficient of variation (%); DM = dry matter; OM = organic matter; CP = crude protein; EE = ethereal extract; NDF = neutral detergent fiber; TC = total carbohydrates; NFC = non-fibrous carbohydrates; TDN = total digestible nutrients.

The response of nutrient digestibility, including DM, OM, CP, and ether extract (EE), varied with the incorporation of protected fat. The digestibility of EE improved, increasing from 0.88 to 0.96 as the energy level of the diets increased, indicating enhanced utilization of fats from diets with higher energy content. However, the digestibility coefficients for DM, OM, CP, and NDF were not affected, suggesting a stable overall nutrient absorption capacity across different lipid levels (Table 2). Total digestible nutrient (TDN) digestibility increased linearly with dietary energy levels, as described by the equation $Y = -0.595 + 0.799X$ ($R^2 = 0.20$, $P < 0.05$), reaching 0.722 kg kg^{-1} at 2.9 Mcal ME kg^{-1} DM. Non-fibrous carbohydrate (NFC) digestibility, in contrast, decreased linearly ($Y = 1.933 - 0.412X$; $R^2 = 0.27$, $P < 0.05$), with the lowest value (0.738 kg kg^{-1}) recorded at 2.9 Mcal ME kg^{-1} DM, possibly due to improved fat utilization.

At 90 days into lactation, blood cholesterol levels increased with dietary energy levels, following the equation $Y = -418.80 + 196.40X$ ($R^2 = 0.46$, $P < 0.05$), with values ranging from 88.00 mg dL^{-1} (2.6 Mcal ME kg^{-1} DM) to 148.80 mg dL^{-1} (2.9 Mcal ME kg^{-1} DM). Triglycerides and glucose levels remained statistically unchanged ($P>0.05$). This suggests that while cholesterol levels in Saanen goats are sensitive to changes in dietary energy from protected fat, triglycerides and glucose concentrations are not markedly affected under the conditions of this study (Table 3).

Table 3
Blood biochemical profile (mg dL^{-1}) of Saanen goats at 90 days in milk receiving diets with increasing energy levels achieved by adding protected fat

Variable	Diets (Mcal of EM kg^{-1} of DM)				Regression equation; R^2		CV (%)
	2.6	2.7	2.8	2.9			
Cholesterol	88.00	117.20	131.20	148.80	Y = -418.80+196,40X;	0.46	21.61
Triglycerides	19.40	23.80	19.00	22.40	Y = 21.15;	ns[1]	22.59
Glucose	57.20	53.40	55.40	59.80	Y = 56.45;	ns	8.5
Urea	43.89	44.16	46.63	53.08	Y = 33.686+30.045X;	020	15.24

[1]ns = $P>0.05$; R^2 = coefficient of determination; CV = coefficient of variation (%).

In Table 3, plasma urea levels in Saanen goats during lactation are shown to increase in response to diets with progressively higher energy content. This reveals a clear trend of rising urea concentrations in the blood as the energy level in the diet increases from 2.6 to 2.9 Mcal ME•kg^{-1} DM, as indicated by the linear regression model ($Y = 33.686+30.045X$; $R^2=0.20$).

Table 4 shows the impact of dietary energy levels on milk yield and quality post-21 days in milk. Initially, from 22 to 60 days, milk yield positively correlated with enhanced dietary energy ($Y = -3.76 + 2.71X$; $R^2 = 0.24$), inversely affecting protein content ($Y = 46.31 - 6.82X$; $R^2 = 0.13$). Fat, total solids, and somatic cell scores were not affected by treatments. In the subsequent 61 to 120 days, milk yield demonstrated a quadratic response to dietary energy ($Y = -147.64 + 106.76X - 18.85X^2$; $R^2 = 0.56$), with significant linear responses observed in milk fat and lactose content to dietary energy levels. Protein content decreased linearly ($Y = 46.66 - 6.83X$; $R^2 = 0.18$), whereas total solids showed a quadratic response ($Y = -1,453.60 + 1125.82X - 201.09X^2$; $R^2 = 0.20$). Somatic cell scores were unaffected. From 121 to 180 days, milk yield's relationship with diet energy also followed a quadratic pattern ($Y = -217.60+157.38X-28.03X^2$; $R^2=0.62$). Both milk fat and protein contents were significantly influenced, emphasizing the direct impact of diet energy variations on milk composition during this phase. Lactose, total solids, and somatic cell scores showed no significant differences across energy levels.

Table 5 describes the milk fatty acid composition at 90 days in milk. The inclusion of protected fat in the diets altered the fatty acid profile. Lauric acid (C12:0) decreased from 3.31 g•100^{-1} of fatty acids in the diet with 2.6 Mcal ME•kg^{-1} DM to 1.65 g•100^{-1} in the diet with 2.9 Mcal ME•kg^{-1} DM ($Y = 17.35 - 5.45X$; $R^2 = 0.72$; $P < 0.05$). Myristic acid (C14:0) and palmitic acid (C16:0) did not exhibit significant variations across treatments ($P > 0.05$). Stearic acid (C18:0) increased from 14.14 g•100^{-1} at 2.6 Mcal ME•kg^{-1} DM to 20.51 g•100^{-1} at 2.9 Mcal ME•kg^{-1} DM ($Y = -41.19 + 21.29X$; $R^2 = 0.52$; $P < 0.05$). Conjugated linoleic acid (CLA; C18:2n6c) followed a quadratic response ($Y = 426.95 - 318.30X + 59.60X^2$; $R^2 = 0.55$; $P < 0.05$), reaching its highest concentration at 5.31 g•100^{-1} at 2.67 Mcal ME•kg^{-1} DM.

Additionally, long-chain fatty acids exhibited varied responses. Linoleic acid (C18:3n3) increased linearly with increasing energy levels in the diet ($p < 0.05$), while arachidonic acid (C20:1) showed a quadratic response ($p < 0.05$), reaching a peak at 2.9 Mcal ME kg^{-1} DM. Short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA) were negatively influenced by the addition of protected fat, decreasing linearly ($p < 0.05$) with increasing energy levels. In contrast, long-chain fatty acids (LCFA) increased linearly ($p < 0.05$), while saturated fatty acids (SFA) were not significantly affected ($p > 0.05$). Polyunsaturated fatty acids (PUFA) and the omega-6 to omega-3 ratio (n6:n3) showed a significant quadratic response ($p < 0.05$), peaking at 2.9 Mcal ME•kg^{-1} DM.

Table 4
Milk yield and composition after 21 days in milk in Saanen goats receiving diets with increasing energy levels achieved by adding protected fat

Variable	Diets (Mcal ME•kg^{-1} DM)				Regression equation; R^2		CV (%)
	2.6	2.7	2.8	2.9			
22 to 60 days in lactation							
MP[1]	3.19	3.82	3.55	4.16	Y = -3.76+2.71X;	0.24	15.71
Fat[2]	35.99	37.29	37.98	38.76	Y = 37.98;	ns[4]	14.64
Protein[2]	27.97	27.51	28.29	27.35	Y = 46.31-6.82X;	0.13	9.48
Lactose[2]	42.31	43.68	42.67	43.46	Y = 29.40+5.16X	0.17	4.05
Total Solids[2]	112.92	115.77	115.85	116.73	Y = 115.32	ns	6.07
SCS[3]	5.35	5.01	6.20	5.44	Y = 5.50	ns	26.45
61 to 120 days in lactation							
MP	2.48	3.29	3.37	3.47	Y = -147.64+106.76X-18,85X^2;	0.56	11.72
Fat	35.93	38.45	39.72	41.39	Y = -19.52+21.46X;	0.19	13.81
Protein	28.49	27.81	28.22	27.17	Y = 46.66-6.83X;	0.18	7.67
Lactose	42.33	43.15	42.69	43.89	Y = 32.02+4.12X;	0.08	3.81
Total Solids	114.74	117.71	118.96	120.85	Y = -1453.60+1125.82X-201.09X^2;	0.20	5.96
SCS	5.63	5.65	6.30	6.17	Y = 5.92;	ns	23.69
121 to 180 days in lactation							
MP	2.06	3.04	3.19	3.07	Y = -217.60+157.38X-28.03X^2;	0.62	13.98
Fat	34.19	36.64	40.77	40.43	Y = -19.49+20.94X;	0.30	11.14
Protein	28.08	27.44	27.75	26.52	Y = 49.99-8.22X;	0.20	8.84
Lactose	41.39	42.09	40.45	42.68	Y = 41.59;	ns	4.73
Total Solids	112.21	114.84	117.62	118.37	Y = 115.88;	ns	5.67
SCS	6.25	6.58	6.52	6.35	Y = 6.41;	ns	18.19

[1]Milk yield (kg/day); [2]g/kg; [3]Somatic cell score; [4]ns = P>0.05; R^2 = coefficient of determination; CV = coefficient of variation (%).

Table 5
Fatty acid profile (g 100 g^{-1} of fatty acids) in milk at 90 days in milk in Saanen goats receiving diets with increasing energy levels achieved by adding protected fat

Variable	Diets (Mcal ME•kg^{-1} DM)				Regression equation; R^2		CV (%)
	2.6	2.7	2.8	2.9			
C4:0	3.01	5.39	4.86	3.41	Y = 4.17;	ns[1]	94.60
C6:0	0.71	0.99	0.78	0.50	Y = 0.75;	ns	70.63
C8:0	1.35	1.55	1.18	0.75	Y = 1.21;	ns	54.11
C10:0	7.12	6.62	5.49	3.67	Y = 37.29-11.48X;	0.30	37.39
C12:0	3.31	2.49	2.03	1.65	Y = 17.35-5.45X;	0.72	17.19
C13:0	0.09	0.05	0.03	0.02	Y = 0.67-0.23X;	0.34	81.27
C14:0	10.32	8.10	7.04	6.45	Y = 42.85-12.68X;	0.77	9.18
C15:0	0.67	0.52	0.23	0.21	Y = 5.06-1.69X;	0.28	80.36
C16:0	31.92	27.99	28.07	29.55	Y = 29.38;	ns	9.25
C18:0	14.14	16.30	18.47	20.51	Y = -41.19+21.29X;	0.52	14.81
C18:1n9t	0.86	4.00	6.22	6.76	Y = -50.26+19.90X;	0.60	42.33
C18:1n9c	22.25	20.94	20.76	18.33	Y = 20.57;	ns	21.87
C18:2n6c	2.15	2.52	2.55	5.31	Y = 426.95-318.30X+59.60X^2;	0.55	37.79
C18:3n6	0.02	0.01	0.02	0.01	Y = 0.01;	ns	63.58
C18:3n3	0.12	0.13	0.17	0.28	Y = -1.28+0.53X;	0.38	46.44
C20:1	0.20	0.29	0.30	0.78	Y = 68.31-51.24X+9.63X^2;	0.48	62.88
Others[2]	1.76	2.10	1.79	1.83	Y = 1.87		-
SCFA	5.07	7.94	6.81	4.65	Y = 6.12;	ns	82.02
MCFA	21.97	18.24	15.28	12.37	Y = 104.32-31.77X;	0.67	16.44
LCFA	72.96	73.82	77.91	82.97	Y = -16.98+34.15X;	0.25	9.58
SFA	73.34	71.15	69.03	67.71	Y = 70.31;	ns	8.55
MUFA	24.22	25.94	27.97	26.38	Y = 26.13;	ns	22.22
PUFA	2.45	2.92	3.00	5.91	Y = 437.21-326.41X+61.25X^2;	0.55	35.80
n3	0.13	0.18	0.18	0.29	Y = -1.11+0.48X;	0.40	35.45
n6	2.28	2.65	2.64	5.39	Y = 426.95-318.02X+59.52X^2;	0.53	36.98
n6:n3	20.23	15.20	16.47	18.61	Y = 17.63;	ns	37.57

[1]ns = P > 0.05; [2]other fatty acids; R^2= coefficient of determination; CV = coefficient of variation (%); SCFA = short-chain fatty acids (C4:0+C6:0+C8:0); MCFA = medium-chain fatty acids (C10:0 to C15:1); LCFA = long-chain fatty acids (longer than C16:0); SFA= saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n3 = omega-3 fatty acids; n6 = omega-6 fatty acids.

Discussion

In this study, we investigated the effects of varying dietary energy levels, achieved through protected fat supplementation with Lactoplus®, on the performance, milk composition, and metabolic health of lactating Saanen goats. By integrating four distinct energy levels (2.6, 2.7, 2.8, and 2.9 Mcal ME•kg^{-1} DM) while maintaining consistent protein content, we aimed to explore the nuanced impacts of dietary energy modulation. This research aligns with broader efforts to enhance dairy production and product quality through optimized feeding strategies, addressing a significant gap in dairy goat nutrition. Furthermore, these findings support previous observations by Parodi (2009), who highlighted the role of dietary strategies in improving milk lipid composition, particularly through increased bioavailability of conjugated linoleic acid (CLA) and long-chain unsaturated fatty acids, which have well-established health benefits for consumers.

The inclusion of protected fats in ruminant diets modulates lipid metabolism by influencing ruminal biohydrogenation, lipid absorption, and milk fat synthesis (Lourenço et al., 2010). Microbial lipolysis in the rumen releases free fatty acids from dietary lipids, which can undergo biohydrogenation, reducing the proportion of unsaturated fatty acids available for absorption (Bauman & Griinari, 2003). However, protected fats bypass this process, allowing a greater proportion of unsaturated fatty acids to reach the small intestine, where they are absorbed and incorporated into milk fat (Cozma et al., 2013). Parodi (2009) further emphasized that these changes in lipid composition are critical, as long-chain fatty acids, particularly omega-3 and CLA, contribute to improved lipid metabolism and may enhance the functional properties of milk.

Our dietary strategy, centered on the supplementation of Lactoplus®, allowed us to assess the direct contributions of dietary energy to lactation performance and health outcomes. The ability of protected fat sources to evade ruminal biohydrogenation increases the availability of unsaturated fatty acids for absorption, where they can be incorporated into circulating lipoproteins and utilized by the mammary gland for milk fat synthesis (Lourenço et al., 2010). Bauman and Griinari (2003) argued that lipid absorption efficiency depends not only on biohydrogenation escape but also on interactions between dietary lipid intake and endogenous fatty acid metabolism, contributing to shifts in the milk fatty acid profile. These authors found that protected fat supplementation shifts the milk fatty acid profile by increasing long-chain fatty acids while reducing *de novo* synthesized short- and medium-chain fatty acids, highlighting the role of dietary interventions in regulating milk composition.

The impact of dietary interventions on milk composition is well-documented. Our results align with those of Titi (2011), who demonstrated that dietary fat supplementation enhances the production and composition of milk in Shami goats. Similarly, Del Valle et al. (2021) investigated the effects of combining calcium salts of soybean oil fatty acids and rumen degradable protein levels in lactating cows, further emphasizing the role of dietary strategies in optimizing milk yield and composition.

The observed modifications in milk fatty acid composition reinforce the importance of tailored feeding strategies. Supplementation with protected fat significantly increased the proportion of long-chain fatty acids in milk while reducing short-chain fatty acids. This shift can be explained by the preference of the mammary gland for circulating preformed fatty acids over *de novo* synthesized fatty acids, as suggested by Lourenço et al. (2010). Cozma et al. (2013) further emphasized that the uptake of circulating lipids by the mammary gland is a major determinant of milk lipid composition, a mechanism that explains the changes observed in our study.

Additionally, as noted by Bauman and Griinari (2003), the presence of preformed fatty acids in circulation can downregulate the synthesis of short- and medium-chain fatty acids, redirecting metabolic precursors toward alternative pathways. The influence of biohydrogenation intermediates, such as trans-10, cis-12 CLA, on the inhibition of *de novo* fatty acid synthesis in the mammary gland further underscores the complexity of lipid metabolism in ruminants. Our findings suggest that supplementation with protected fat may have influenced this process by promoting the incorporation of unsaturated fatty acids and reducing the synthesis of short-chain fatty acids, which aligns with previous literature

Beyond compositional changes in milk fat, dietary energy levels also influenced metabolic markers in lactating goats. The observed linear increase in blood cholesterol levels with higher dietary energy demonstrates the intricate balance between lipid supplementation and lipid metabolism. These results corroborate findings from Beam and Butler (1998), who emphasized the need to optimize dietary energy levels to support metabolic health and milk production. Additionally, the quadratic response in milk production and the significant increases in milk fat and lactose content highlight the critical role of dietary energy balance in maximizing production efficiency. Parodi (2009) reinforced the role of dietary energy in regulating lipid metabolism and cholesterol synthesis, stressing the need for precision in dairy nutrition management.

The interplay between dietary energy and nitrogen metabolism was also evident in our study. The effect of lipid supplementation on nitrogen utilization is complex, as it may influence microbial protein synthesis and nitrogen retention in the rumen. Doreau and Ferlay (1995) observed that dietary lipids tend to decrease ammonia concentration in the rumen without significantly altering duodenal non-ammonia nitrogen flow, indicating that microbial protein synthesis efficiency might be maintained or even enhanced under specific conditions. Similarly, Del Valle et al. (2021) found that fat supplementation improved microbial protein synthesis efficiency, although it did not significantly affect microbial nitrogen flow. These findings suggest that lipid sources, when carefully selected, could support nitrogen utilization without compromising microbial growth. Given the known impact of lipid supplementation on ruminal metabolism, it is plausible that higher dietary lipid inclusion may have influenced nitrogen utilization for microbial protein synthesis. Some studies suggest that unsaturated fatty acids can modify microbial activity, altering nitrogen metabolism pathways and leading to variable effects on microbial efficiency

and nitrogen losses (Del Valle et al., 2021). Furthermore, increased dietary fat can lead to reduced ammonia-N concentrations in the rumen, potentially decreasing nitrogen losses through urine while enhancing microbial nitrogen assimilation (Rennó et al., 2014). Del Valle et al. (2021) found that dietary fat supplementation could influence ruminal fermentation, affecting microbial protein synthesis and nitrogen metabolism. Additionally, Andjelić et al. (2022) reported that variations in metabolic markers, including urea levels, could reflect broader shifts in energy and nitrogen balance, particularly in early lactation. These findings reinforce the importance of considering the metabolic trade-offs associated with dietary lipid inclusion when formulating feeding strategies for lactating ruminants. Higher dietary energy intake led to an increase in plasma urea levels, reflecting complex interactions between protein utilization efficiency and nitrogen balance (Reynolds et al., 2001). Understanding these metabolic interactions is crucial for designing feeding strategies that optimize production while mitigating environmental nitrogen losses.

Finally, our results have broader implications for milk quality and human health. The increase in long-chain fatty acids at higher energy levels enhances the nutritional profile of milk, particularly by increasing unsaturated fatty acids beneficial to cardiovascular health (Cozma et al., 2013). At the same time, the reduction in short- and medium-chain fatty acids may contribute to lowering levels of hypercholesterolemic saturated fats in milk, as suggested by studies on lipid metabolism in ruminants (Bauman & Griinari, 2003). Parodi (2009) underscores the potential of dairy fat to positively contribute to human health when milk composition is optimized through strategic dietary interventions.

Future research should explore the long-term effects of different lipid sources on milk composition and metabolic responses in dairy goats. Investigating the interactions between dietary fat, rumen microbiota, and systemic metabolism will provide valuable insights for the development of sustainable and efficient dairy production systems. These findings contribute to an expanding body of knowledge aimed at optimizing the nutritional value of milk while ensuring production efficiency and metabolic health in dairy animals.

Conclusion

Increasing dietary energy levels through calcium salts of fatty acids (CSFA) supplementation at 2.6 to 2.9 Mcal ME•kg^{-1} DM improved nutrient utilization efficiency, altered the fatty acid profile of milk, and enhanced milk production in Saanen goats without negatively affecting dry matter intake or metabolic parameters. The supplementation led to a higher proportion of long-chain fatty acids in milk, while reducing the synthesis of short- and medium-chain fatty acids. Additionally, dietary energy levels were positively correlated with blood cholesterol levels and nitrogen metabolism, indicating metabolic adjustments in response to increased energy intake.

These results suggest that CSFA supplementation is a viable strategy to optimize milk yield and composition in Saanen goats without compromising metabolic health. Future studies should explore the

long-term effects of different lipid sources on milk composition and metabolic responses to further refine feeding strategies for sustainable dairy goat production.

Conflict of interest

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

Acknowledgements

The authors would like to thank the support of the Brazilian National Council for Scientific and Technological Development (CNPq), National Institute of Science and Technology for the Dairy Production Chain (INCT–Leite, UEL/UEM), Araucária Foundation, and Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES).

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